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Fast-scanning reflection-mode integrated photoacoustic and optical-coherence microscopy

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ABSTRACT

We previously demonstrated that multimodal microscopy combining photoacoustic microscopy and optical coherence tomography can provide comprehensive insight into biological tissue at μm-level resolution by exploiting both optical absorption and scattering contrasts. Recently, we have developed a second-generation integrated photoacoustic and optical-coherence microscope, which can potentially be adapted for clinical applications. In this new system, we can perform photoacoustic and optical-coherence imaging simultaneously at a speed of 5,000 A-lines per second with real-time on-screen display. Also, both modalities now work in reflection mode instead of transmission mode, allowing easy access to various anatomical locations of interest. Imaging of skin and eye has been demonstrated in living small animals.

Key words: Multimodal imaging, Photoacoustic imaging, Optical coherence tomography, Dermatology, Ophthalmology.

1. INTRODUCTION

A key motivation for imaging tissue using light is that it can provide various contrasts. To exploit this advantage, we proposed a new multimodal microscopy named integrated photoacoustic and optical coherence microscopy [1]. It combines two naturally complementary optical imaging techniques, photoacoustic microscopy (PAM) and optical coherence tomography (OCT) in a single system. PAM is predominantly sensitive to optical absorption. By detecting hemoglobin, it can visualize 3D microvasculature noninvasively with μm-order spatial resolution [2, 3]. Also using spectroscopic information, important hemodynamic parameters, such as the total hemoglobin concentration and the blood oxygen saturation can be calculated by PAM [2]. OCT exploits optical backscattering and provides high-resolution *in vivo* biopsy of tissue. Also based on the Doppler principle, OCT can quantify the blood flow [4]. Thus, using endogenous contrasts, an integrated photoacoustic and optical coherence microscope can provide comprehensive information about microcirculation, including the morphology of the three-dimensional microvasculature and its local environment, hematocrit, blood oxygenation, volumetric blood flow, and potential local metabolism rate, etc.

Our first-generation system [1], where the photoacoustic subsystem worked in transmission mode, targeted at applications in semi-transparent animal models, such as a mouse ear. Here, we report our recent development of a second-generation fast-scanning reflection-mode integrated photoacoustic and optical coherence microscope. Also, we demonstrated its potential applications in dermatology and ophthalmology in living mice.

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2. SYSTEM SET-UP

The schematic of our second-generation integrated photoacoustic and optical-coherence microscope is shown in Fig. 1. Both PAM and OCT work in reflection mode, allowing us probe various tissue of interest. The OCT subsystem adopts a typical fiber-based spectral-domain set-up. The photoacoustic signal is excited by a diode-pumped Nd:YVO_4 laser at 532 nm. The light beams illuminating the two subsystems are combined using a dichroic mirror, and focused into the sample. The generated photoacoustic wave and the backscattered light are separated by a home-made light-sound splitter. OCT senses the backscattered light using a custom-made spectrometer, while PAM detects the photoacoustic wave using a cylindrically focused ultrasonic transducer with a center frequency of 25 MHz. A B-scan image is obtained by scanning the optical focus using a galvanometer along the acoustic focal line (or the *x*-axis). To acquire a volumetric dataset, the imaging probe enclosed in the gray dashed rectangle is loaded on a linear stage and scanned along the *y*-axis.

The lateral resolutions of both the PAM and OCT images are determined by the optical focal widths, estimated to be ~3.5 μm by imaging a USAF-1951 resolution test chart. By combining optical and mechanical scanning scheme, the current system achieves a good balance between photoacoustic detection sensitivity and imaging speed, given the limited photoacoustic excitation energy. Currently, using safe exposure permitted by the ANSI laser safety standards, we are able to acquire both PAM and OCT images in an interlaced manner at a speed of 5,000 A-lines/second, while still maintaining the sensitivity to detect single capillary. B-scan images with 800 A-lines can be repeated at 6 frames/second. A typical volumetric dataset consisting of 800×800 A-lines takes ~2 minutes to acquire. Also, this new system features real-time on-screen display of photoacoustic and optical-coherence images. In future clinical applications, it can guide physicians to quickly identify locations of interest, and allows them to interpret the images on-line.

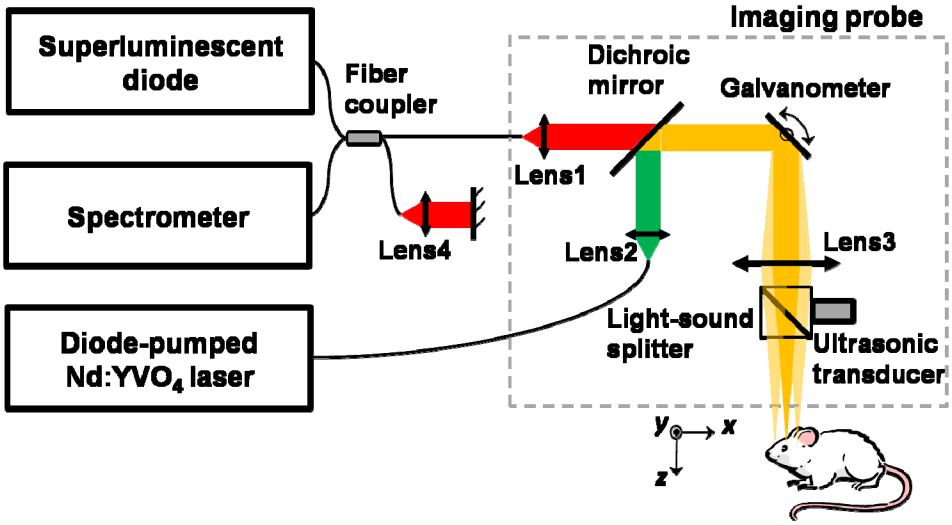

Fig. 1. Schematic of the fast scanning reflection-mode integrated photoacoustic and optical coherence microscope.

3. RESULTS

3.1 Application in dermatology

Fig. 2 shows a piece of mouse scalp imaged *in vivo* by the current system. Compared to the mouse ear model, it is more close to native human skin. Both PAM and OCT could image through the complete skin layer providing complementary information. PAM depicts the 3-D morphology of cutaneous microvasculature down to the capillary level (Figs. 2a, d and g). OCT help us differentiate the epidermis, dermis, subcutaneous skull, and the hair follicles (Figs. 2b, e and h). The current system automatically registered the dual-modality images. From the composite images (Figs. 2c, f and i), we can clearly visualize the laminar structure of micro-vessels in skin, and identify the exact location of each single vessel.

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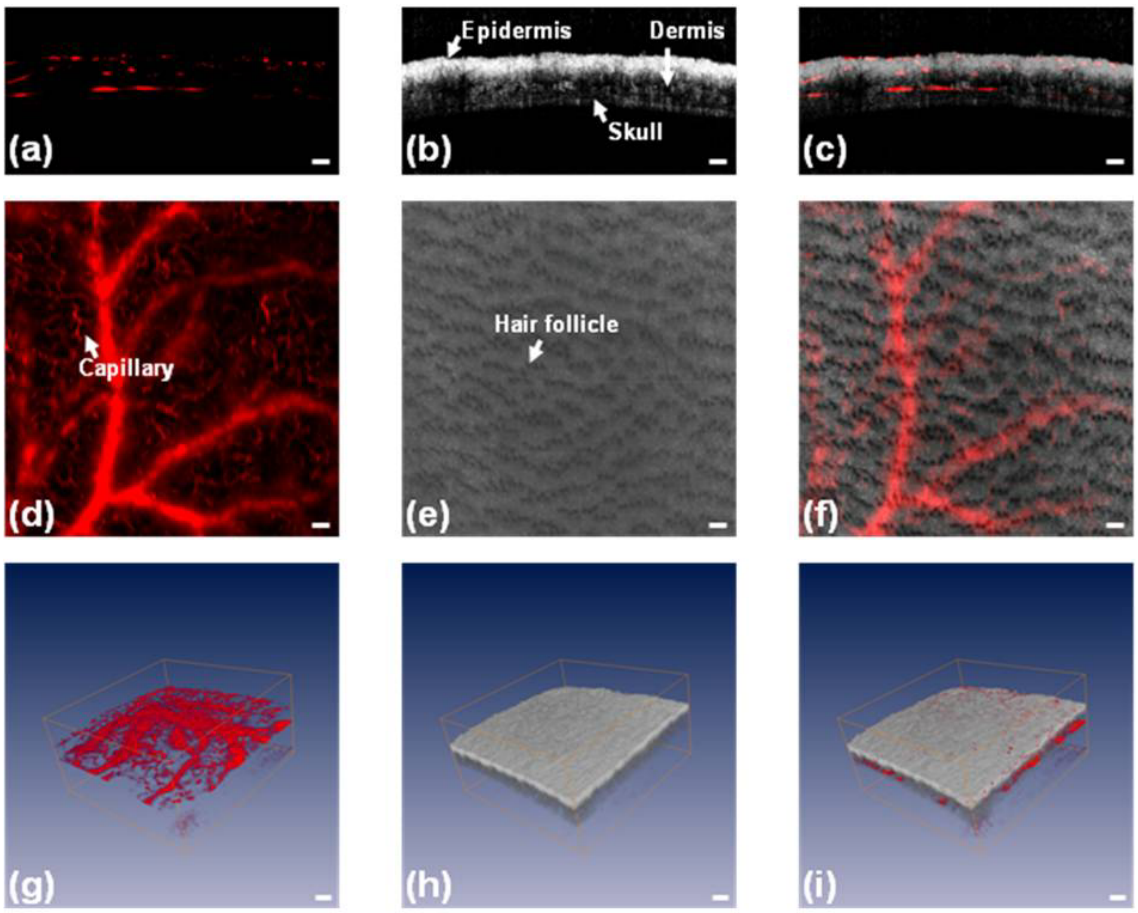

Fig. 2. *In vivo* images of mouse scalp. (a) A PAM B-scan. (b) An OCT B-scan. (c) A composite dual-modality B-scan. (d) A PAM projection image. (e) An OCT projection image. (f) A composite dual-modality projection image. (g) 3-D microvasculature imaged by PAM. (h) 3-D soft tissue imaged by OCT. (i) A composite 3-D visualization. PAM contrast: red. OCT contrast: gray scale. Scale bar: 100 μm.

3.2 Application in ophthalmology

OCT is becoming a major clinical tool in ophthalmological diagnosis. We believe that, by combing OCT with photoacoustic imaging, we can provide physicians with additional useful information, such as microvascular deformation and hemodynamic function. As a demonstration, we imaged the anterior segment of a Swiss-Webster mouse *in vivo* (Fig. 3). While OCT revealed the location and shape of the cornea, iris, and the front part of the lens, PAM mapped micro-vessels in the eye, most of which were located on the iris. We predict that, with further development of functional imaging capabilities, integrated photoacoustic and optical coherence microscopy will impact the understanding and diagnosis of a wide range of eye diseases, such as glaucoma, diabetic microangiopathy, and ocular tumor, which are previously evaluated using fluorescence angiography with exogenous contrast agents.

4. SUMMARY

We have developed a second-generation reflection-mode dual-modality microscope integrating PAM and OCT. It is currently able to acquire dual-modality images at an accelerated speed of 5,000 A-lines/second, while still maintaining the sensitivity to detect single capillary. With the technical development, we further demonstrated non-invasive imaging of skin and eye in living mice. We anticipate that current system can be translated to clinical applications in dermatology directly, and in ophthalmology with some adaption. We predict that an OCT subsystem will be an indispensable part in future commercial photoacoustic imaging device for applications in the optical ballistic regime.

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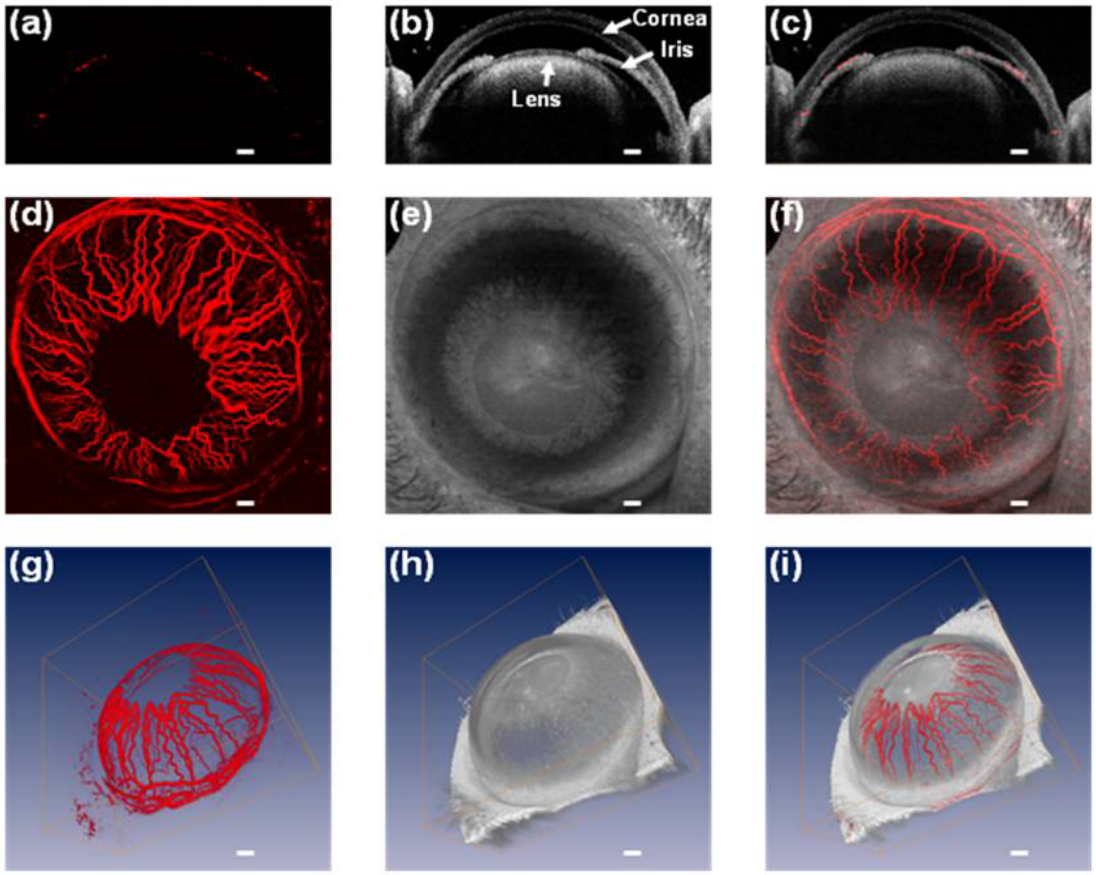

Fig. 3. *In vivo* images of the anterior segment of a mouse eye. (a) A PAM B-scan. (b) An OCT B-scan. (c) A composite dual-modality B-scan. (d) A PAM projection image. (e) An OCT projection image. (f) A composite dual-modality projection image. (g) 3-D microvasculature imaged by PAM. (h) 3-D soft tissue imaged by OCT. (i) A composite3-D visualization. PAM contrast: red. OCT contrast: gray scale. Scale bar: 100 μm.

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